FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

SUPPORTING DOCUMENT 3

APPLICATION A1005
EXCLUSIVE USE OF TONALIN® CLA AS A NOVEL FOOD

Effect of Conjugated Linoleic Acid on Glucose Homeostasis

December 2010

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Glossary

BFM	Body fat mass
BMI	Body mass index
BP	Blood pressure
BW	Bodyweight
CLA	Conjugated linoleic acid
FSANZ	Food Standards Australia New Zealand
HbA_{1c}	Glycosylated haemoglobin
HOMA or HOMA-IR	Homeostasis Model Assessment of Insulin Resistance
ISI	Insulin Sensitivity Index
ITT	Intention to treat
LBM	Lean body mass
LCD	Low calorie diet
ND	No data
NS	Not statistically significant
OGIS	Oral glucose insulin sensitivity
OGTT	Oral glucose tolerance test
QUICKI	Quantitative Insulin Sensitivity Check Index
SBP	Systolic blood pressure
SD	Standard deviation
SEM	Standard error of the mean
TAG	Triacylglycerol
TG	Triglyceride

Summary

This assessment includes 20 human studies of CLA with *c9,t11* and *t10,c12* isomers in a 1:1 ratio. Eight studies reported testing the effect of CLA on glucose homeostasis as part of the study objective. The remaining twelve studies had other primary objectives, such as testing the effect of CLA on body weight, but reported measures of fasting glucose or insulin concentrations as secondary outcomes.

Two studies assessed the effect of CLA on insulin sensitivity using a clamp technique that is recognised as the 'gold standard' method for directly determining insulin sensitivity in humans. Two studies used oral glucose tolerance tests to measure glucose tolerance directly. The majority of human studies assessed fasting blood insulin and glucose concentrations, and sometimes estimated insulin sensitivity from these using surrogate indices. The surrogate indices have been validated against the clamp technique.

The majority of studies in the assessment reported non-significant results, including the two studies that employed the 'gold standard' clamp technique. Two studies reported significant adverse effects of CLA on glucose homeostasis and these two studies were of subjects with type 2 diabetes. Both studies of diabetics used surrogate indices but only one used oral glucose tolerance tests.

Indicators of glucose homeostasis may respond differently to interventions, depending on the health status of the subjects. However, the description of participants in the studies was not adequate for clearly dividing the studies into groups with diabetes, impaired glucose metabolism, metabolic syndrome or normal metabolism.

The variable design of the small number of studies in this assessment limits comparison of results across studies. The inconsistent, but small, results across the studies may relate to subject characteristics and/or study design.

The two studies of CLA in children and adolescents do not allow any conclusions to be drawn for this group.

The available data raises questions but do not permit a conclusion about the effect of CLA on glucose homeostasis in the general population. Two well conducted studies raise safety concerns about the effects of CLA on people with type 2 diabetes.

1. Introduction

The Applicant, Cognis GmbH, is seeking to amend Standard 1.5.1 – Novel Foods of the *Australia New Zealand Food Standards Code* (the Code) to approve the use of a chemically defined mixture of approximately equal amounts of the *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA in the form of triglyceride esters. They recommend 1.5 g Tonalin® CLA be added to individual serves of food with a recommended daily consumption of 4.5 g Tonalin® CLA.

The isomers of CLA of most interest in this assessment are those where the double bonds are in the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 configuration; in this report the shorthand for these will be *c*9,*t*11 and *t*10,*c*12 respectively. Tonalin® consists of an approximate one-to-one ratio of *c9,t11* and *t10,c12* and therefore the assessment focuses on studies that tested CLA in this form. Studies of other CLA isomer preparations have also been published. The results of six of these studies are discussed briefly in Appendix 2, but conclusions in this assessment are not based upon those results.

This report reviews published human studies reporting the effects of CLA (*c9,t11* and *t10,c12* in one-to-one ratio) on glucose homeostasis.

1.1 Glucose homeostasis

Insulin is produced by the pancreas to facilitate glucose absorption. Glucose intolerance[1] and poor insulin sensitivity or insulin resistance[2] are associated with an increased risk of type 2 diabetes and cardiovascular disease (NZGG, 2003).

Animal studies have suggested that CLA can improve glucose metabolism (Risérus *et al.,* 2004). Studies in humans have shown mixed results in relation to insulin resistance and glucose sensitivity, with some studies suggesting that some isomers improve glucose metabolism and others suggesting CLA leads to insulin resistance (Park, 2009).

1.1.1 The metabolic syndrome

The metabolic syndrome is a cluster of risk factors for heart disease. "Definitions of the metabolic syndrome have varied over time and in the past it has been referred to as 'Syndrome X', the 'Deadly Quartet' or 'Insulin Resistance Syndrome'" (IDF, 2006). For the purposes of this assessment, FSANZ will adopt the worldwide definition of the metabolic syndrome for use in clinical practice by the International Diabetes Federation (IDF, 2006). According to the IDF definition, for a person to be defined as having the metabolic syndrome, they must have:

- **Central obesity** defined as waist circumference ≥ 94 cm for Europid men and ≥ 80 cm for Europid women, with ethnicity specific values for other groups, plus any two of the following four factors
    - **Raised TG level** defined as ≥ 150 mg/dL (1.7 mmol/L), or specific treatment for this lipid abnormality

[1] **Impaired fasting glucose** is a condition where fasting glucose levels are higher than normal but lower than diagnostic levels for diabetes mellitus.

[2] "**Insulin resistance** occurs when cells in the body (liver, skeletal muscle and adipose tissue) become less sensitive and eventually resistant to insulin" (IDF, 2006). Universal cut-off points for insulin resistance have not been defined due to lack of a standardised insulin assay (Muniyappa *et al*., 2008).

- **Reduced HDL cholesterol** defined as < 40 mg/dL (1.03 mmol/L) in males and < 50 mg/dL (1.29 mmol/L) in females, or specific treatment for this lipid abnormality

- **Raised blood pressure** defined as systolic BP ≥ 130 or diastolic BP ≥ 85 mmHG, or treatment of previously diagnosed hypertension

- **Raised fasting plasma glucose** ≥ 100 mg/dL (5.6 mmol/L), or previously diagnosed type 2 diabetes. If above 5.6 mmol/L or 100 mg/dL, OGTT is strongly recommended but is not necessary to define presence of the syndrome.

The International Diabetes Federation acknowledges that the pathogenesis of the metabolic syndrome and each of its components is complex and not well understood. However, insulin resistance is acknowledged as an important causative factor of the metabolic syndrome. People with metabolic syndrome have a fivefold greater risk of developing type 2 diabetes[3]. "Eighty-five per cent of obese[4] individuals have some degree of insulin resistance which can be improved with weight loss" (IDF, 2006).

1.1.2 Parameters under consideration

FSANZ refers to an in-depth review, *Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage*, for the information contained under this sub-section (Muniyappa *et al*., 2008).

The hyperinsulinaemic euglycaemic glucose clamp (clamp technique) is widely accepted as the 'gold standard' for directly determining insulin sensitivity in humans. It requires medical expertise and a clinical setting and is time- and labour-intensive to perform. With insulin being constantly infused (hyperinsulinaemic means above the fasting level), and dextrose being infused at a variable rate to clamp blood glucose concentrations in the normal range (euglycaemic), glucose disposal rate (M) can be determined directly. "The value of M is typically normalised to body weight or fat-free mass to generate an estimate of insulin sensitivity" (Muniyappa *et al.,* 2008).

An insulin sensitivity index (ISI) can be derived from clamp data.

$$ISI_{clamp} = M/(G \times \Delta I)$$

Where M is glucose disposal rate,
G is steady-state blood glucose concentration and
ΔI is difference between fasting and steady-state[5] plasma insulin concentrations.

There are also indirect measures of insulin sensitivity including the commonly used oral glucose tolerance test (OGTT). Fasting subjects consume an oral glucose load (75 g) and blood samples are taken at intervals to measure the body's ability to dispose of the glucose. An OGTT provides information about glucose tolerance but not insulin sensitivity or insulin resistance *per se*.

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[3] Stern M., *et al*., (2004). Does the metabolic syndrome improve identification of individuals at risk of type 2 diabetes and/or cardiovascular disease? *Diabetes Care,* **27**(11): 2676-81. Cited in background to IDF worldwide definition of the metabolic syndrome (IDF, 2006).

[4] Obesity is defined by a body mass index (BMI) ≥ 30kg/m^2. Overweight is defined by a BMI > 25 and <30 kg/m^2.

[5] A 'steady state' refers to conditions when blood concentrations are not changing.

Surrogate indices are often used for measures of insulin resistance. These are derived from fasting glucose and plasma insulin concentrations taken at a single point in time, so they are relatively inexpensive and easy to use in epidemiological studies or controlled trials.

The homeostasis model assessment of insulin resistance (HOMA or HOMA-IR) is a surrogate index.

$$\text{HOMA} = \frac{\left\{\left[fasting\ insulin\left(\frac{\mu U}{ml}\right)\right]\times\left[fasting\ glucose\left(\frac{mmol}{L}\right)\right]\right\}}{22.5}$$

'The product of normal fasting plasma insulin of 5 µU/ml and normal fasting plasma glucose of 4.5 mmol/L is 22.5 (i.e. the denominator in the HOMA equation is a normalising factor). For an individual with normal insulin sensitivity, HOMA = 1' (Muniyappa *et al.,* 2008).

Plasma insulin concentrations do not follow a normal distribution curve[6]. Muniyappa *et al.,* (2008) report that log (HOMA) transforms the skewed distribution, and that log (HOMA) has a stronger linear correlation with direct insulin sensitivity measures (from the clamp technique) than HOMA.

The quantitative insulin sensitivity check index (QUICKI) is another widely used index.

$$\text{QUICKI} = 1/[\log(fasting\ insulin\ \left(\frac{\mu U}{ml}\right) + \log(fasting\ glucose\ \left(\frac{mg}{dL}\right)]$$

Muniyappa *et al*., (2008) report that QUICKI has a substantially better linear correlation with direct insulin sensitivity measures (clamp technique) than HOMA. "QUICKI is proportional to 1/log(HOMA)".

If insulin sensitivity is low then insulin resistance is high; thus a decrease in QUICKI result indicates the same direction of physiological change to an increase in HOMA result. It should be noted that because the formulae are different, even though the two indices are mathematically related, the value calculated for one index cannot be simply inverted to yield the other. However, to generalise, an increase in HOMA (increase in insulin resistance) is an adverse finding, whereas an increase in QUICKI (increase in insulin sensitivity) indicates a favourable finding.

An increase in glycosylated haemoglobin (HbA_{1c}) indicates poor control of blood glucose in diabetics. HbA_{1c} is commonly used in management of type 2 diabetes. The aim is to keep HbA_{1c} levels below 7%. Diabetes management should be reviewed every 3-6 months. Any sustained reduction in HbA_{1c} should be seen as a positive outcome (NZGG, 2003b). The meaningfulness of HbA_{1c} measures taken from non-diabetic subjects is not clear.

2. Methods

Given that reviews of the literature have previously reported mixed results (e.g. Park, 2009), FSANZ has undertaken its own systematic review to analyse the effect of CLA on measures of glucose homeostasis (see Inclusion and Exclusion criteria below).

[6] Data that is normally distributed will follow a bell-shaped curve, where the peak of the curve is at the mean and most of the results cluster symmetrically either side of that mean.

2.1 Literature search strategy

The Applicant provided the bulk of scientific literature reviewed as published peer reviewed papers. The reference lists of the papers provided were searched for further relevant work. PubMed was also searched using the terms: conjugated linoleic acid OR CLA. The following limits were applied to the search: humans, controlled clinical trial. Relevant articles containing information on the effect of CLA on glucose or insulin outcome measures were identified for further review. The search was last run on 31 March 2010. A CLA specific website was also searched http://fri.wisc.edu/clarefs.htm (last accessed on 23 November 2010).

2.2 Inclusion and exclusion criteria

To be considered for detailed evaluation and inclusion in this assessment, human studies were required to:

- Be published as a full report to allow critical evaluation
- At least report that the study had been randomised and double-blinded
- Be controlled – parallel[7] or crossover[8] design
- Include only CLA as the potentially active ingredient in the diet unique to the test group
- Report measures of insulin sensitivity or glucose homeostasis.

Figure 1 provides relevant details of studies excluded from this assessment.

[7] This involves two or more groups run in parallel where one group is given a control and the other(s) the treatment for the duration of the study period. In this design one group acts as a comparison for the other group.

[8] This involves study participants given a treatment or control and then crossing over to the opposite treatment/control; sometimes this involves a period where no treatment/control is administered called a 'washout' period. In this design each participant acts as their own control.

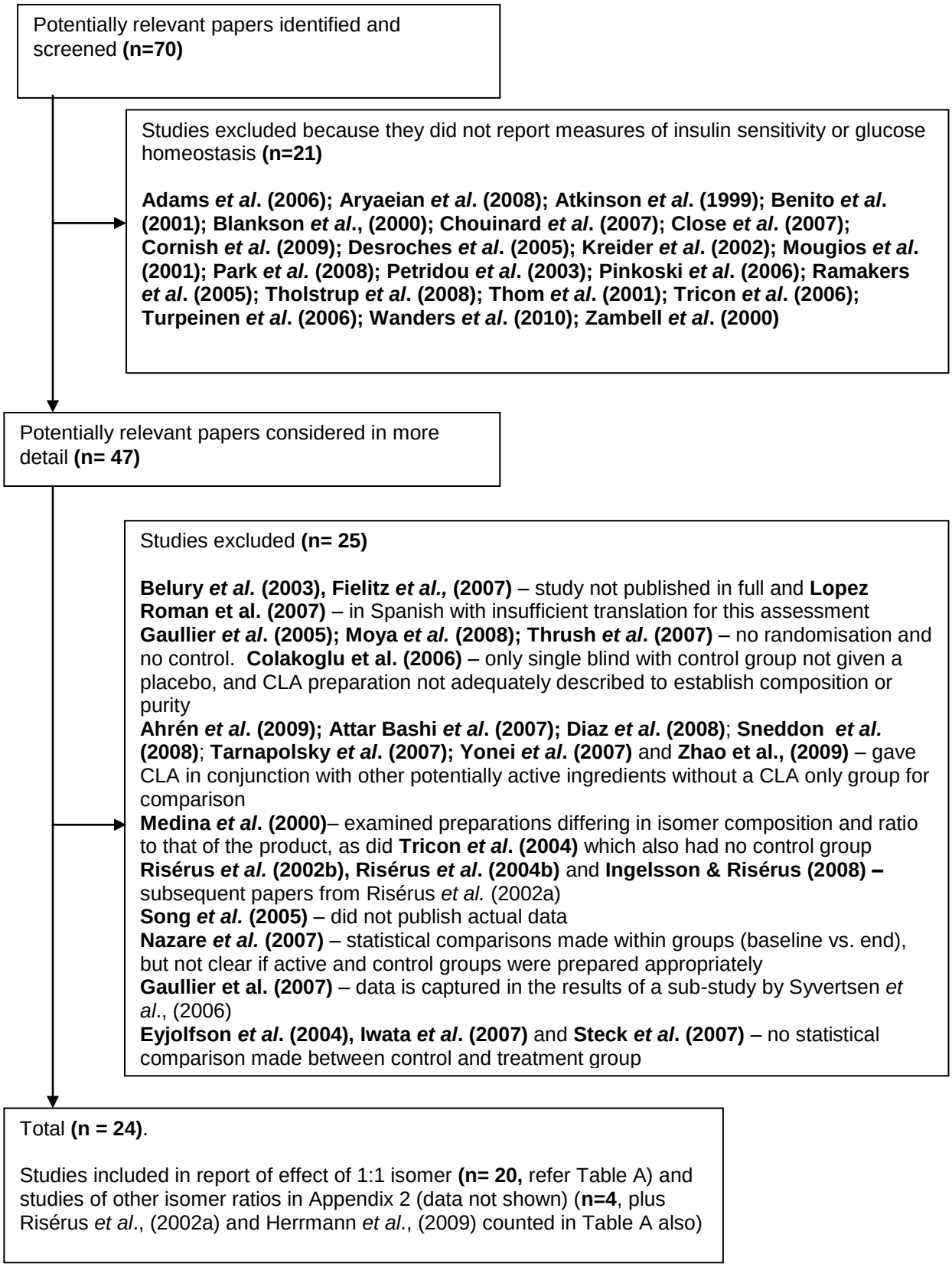

Figure 1: Flow of study consideration and reasons for exclusions in the systematic review of effect of CLA on glucose homeostasis

2.3 Studies identified

Figure 1 summarises the reason for exclusion of 46 of the 70 studies initially identified by the search. The majority of retained studies informed the assessment of the effect of CLA on glucose homeostasis. The majority used a parallel study design. Two studies utilised a crossover design (Norris *et al*., 2009 and Herrmann *et al*., 2009).

2.3.1 Administration and Form of Conjugated Linoleic Acid

Four studies investigated CLA delivered in a food vehicle. Racine *et al.* (2010) delivered CLA via a chocolate milk drink to overweight and obese children and Bonet Serra *et al* (2008) used a yoghurt drink to deliver CLA to adolescents. Laso *et al.* (2006) fed adult participants skim milk supplemented with 3 g CLA while controls were fed the same volume of skim milk with no additions. This study has been excluded from other aspects of FSANZ's assessment of CLA because no fatty acid was given to the control group. In an otherwise ad libitum diet, it is not clear if an additional 3 g fatty acid per day would affect glucose homeostasis in the control group. Laso *et al.* (2006) is included in this assessment, noting the less than ideal control of the intervention. Raff *et al*. (2008) added manufactured CLA of a 1:1 isomeric ratio to low CLA butter as the intervention and gave low CLA butter to the control group.

For all other studies, CLA and placebo were administered in the form of soft gel capsules, which were designed so that CLA and control capsules appeared identical. The purity of the CLA, in terms of the *c9,t11* and *t10,c12* isomers of interest, varied between approximately 70% to above 80%. The balance of capsule weight came from other CLA isomers in varying but minor amounts, and other lipids.

In this report, the dose of CLA, unless otherwise stated, refers to the total daily amount of *c9,t11* and *t10,c12* isomers provided. For example, if study participants were given 4 g of total CLA supplements but *c9,t11* and *t10,c12* in equal proportions totalled 75% of capsule weight (75% purity), then the dose of CLA will be reported in this assessment as 3 g.

2.4 Study limitations and confounding factors

The Applicant wishes to incorporate CLA into foods. Sixteen of the 20 studies that tested CLA using the 1:1 isomer mix of interest provided CLA in capsules (refer Table A). It is not clear whether it is appropriate to extrapolate findings from these studies to CLA when incorporated into foods. The majority of studies reported that participants were asked to consume capsules at meal times. This method may or may not mimic CLA being consumed as part of a complex food matrix, much as it would when incorporated into food directly. The issue of CLA stability in food will not be discussed here.

Studies captured in this assessment were predominantly undertaken using subjects in free-living situations; quite often subjects were advised not to alter their usual eating or physical activity patterns. The outcome measures of interest in this assessment may have suffered confounding. For example, macronutrient intakes and levels of physical activity were often not captured and/or published to facilitate assessment of their potential influence on glucose homeostasis.

Not all studies assessed dietary intakes (refer Table A for study-specific details). In those studies where dietary intakes were recorded, different methods of dietary intake assessment were used.

In some studies, participants were required to maintain diet records, which varied from 3-7 days of recording. This type of recording can impose a high degree of respondent burden so inaccuracies in these records would be expected, as would changes in dietary intake as a result of the burden of keeping a record (Gibson, 2005). Conversely, some studies did not report collecting any data on diet or physical activity. Differences in respondent burden across the studies leading to different behaviours may account for some of the variation in study results. Given the relatively small sample sizes in the available studies, randomisation would not rule out such differences.

The majority of studies report a modified intention-to-treat analysis in which the results of those participants completing each relevant part of the study are included in the statistical analysis. A small number of studies excluded participants with lower compliance. The extent of the dropouts varied across studies (refer Table A). In combination with the small sample sizes in many trials, this may bias the results owing to unbalanced baseline characteristics of the participants.

3. Results

3.1 Analysis and reporting by FSANZ

Table A (appended) provides details about the design of the 1:1 isomer studies, including samples sizes, dropouts, age and physical state of subjects, study duration, CLA dose, type of control and its dose, and a brief report of dietary and physical activity assessment.

Table B (appended) provides details of 1:1 isomer study results, including changes in insulin concentration from baseline (pmol/L), changes in blood glucose concentration from baseline (mmol/L), changes in HbA_{1c} (%) from baseline and changes in HOMA from baseline.

The focus of the analysis is on the change in average measures (means) in the CLA groups relative to the change in the same measures in the placebo groups at the end of the study. A comparison of the effect between treatment and control (i.e. the amount of change) was frequently not reported in studies. As such, the results are confined to the significance of the difference between treatment and placebo rather than the effect size. All results are based on those who completed the study protocol, except where stated otherwise, because not all studies describe the number randomised.

Differences in effect of CLA on glucose homeostasis may depend on a range of variables, including but not limited to age, gender, body composition, physical activity and any underlying disease states of participants. To assist with interpretation and assessment of the available evidence, FSANZ grouped studies as much as possible according to the effect of CLA on glucose homeostasis:

1. In adults with diabetes or the metabolic syndrome

2. In otherwise healthy overweight or obese adults

3. Following initial weight reduction

4. In healthy adults with a normal body weight

5. In overweight and obese children and adolescents.

An explanation of the grouping of studies in tabulated results is provided in Appendix 1.

3.1.1 Overview of key features of 1:1 isomer studies in this assessment

In Section 3.2, results are summarised and presented under the groupings listed above (based primarily on subject characteristics). In this section, key features of study design are further highlighted, such as consideration of study objectives and, in particular, the methods used to measure markers of glucose homeostasis.

This assessment includes 20 studies of CLA isomers in a 1:1 ratio (refer Table A). Fasting in all 20 studies was overnight; the length of fast and any other instructions given to subjects to follow the day or days preceding blood collection varied (data not shown).

Of these 20 studies, seven studies of adults reported that testing insulin action was the main objective or part of the aim of the study (Lambert *et al* 2007, Moloney *et al*., 2004, Norris *et al*, 2009, Risérus *et al*, 2002a, Syvertsen *et al*, 2006, Taylor *et al*, 2006 and Raff *et al.,* 2008). The study of children and adolescents by Bonet Serra *et al*., (2008) also aimed to determine if CLA improved insulin resistance in obese subjects. Of the seven adult studies:

- Two studies used the 'gold standard' clamp technique to directly measure insulin sensitivity (Risérus *et al*., (2002a) and Syvertsen *et al.,* (2006)).

- Two studies performed oral glucose tolerance tests (Lambert *et al.,* (2007) and Moloney *et al*., (2004)). Therefore, these are the only studies investigating insulin action in a dynamic state, which is more likely to mimic physiological conditions such as the body's response to consuming a meal.

- One study used food vehicles to deliver CLA (via butter) (Raff *et al.,* 2008), while the other studies used capsules.

- The subjects in the study by Lambert *et al*., (2007) were exercising and had a healthy body weight. In Raff *et al*., (2008), subjects were lean healthy men and were included if they undertook heavy exercise for no more than 10 hours per week. In the other five studies, subjects were overweight or obese.

- The overweight or obese subjects in Taylor *et al*., (2006) were described as healthy. Syvertsen *et al*., (2006) described healthy obese subjects also, however they report some subjects were insulin resistant at baseline and, going by the authors' exclusion criteria, there may have been diet-controlled type 2 diabetics in that study.

- Subjects in the study by Risérus *et al*., (2002a) had metabolic syndrome and 6 of the 57 subjects had markers indicating mild diabetes (undiagnosed prior to study).

- The studies by Norris *et al.,* (2009) and Moloney *et al*., (2004) involved diabetics; in the former the subjects were taking oral hypoglycaemic medication and had baseline mean fasting glucose concentrations < 5 mmol/L which is within the normal range; while in the latter, the subjects' diabetes was diet-controlled, some were taking anti-hypertensive medication and baseline mean fasting glucose concentrations were 7.3 mmol/L.

The other 12 studies in this assessment collected measures of fasting glucose and/or fasting insulin concentrations as secondary outcome measures (refer Table A). The studies usually had objectives such as testing the effect of CLA on body weight or body composition, and included healthy, overweight and obese subjects. Age varied across studies.

- Three of these twelve studies involved a phase of initial weight loss to investigate body weight regain (Kamphuis *et al.,* (2003), Larsen *et al.,* (2006) and Whigham *et al*., (2004)).

- In three studies, possibly mingled among the reportedly healthy subjects, were subjects with diet-controlled diabetes or indicators of the metabolic syndrome. In these studies, those subjects were not clearly identified (Larsen *et al*., (2006), Laso *et al*., (2007) and Risérus *et al*., (2001)).

- Herrmann *et al.,* (2009) was a crossover study with emphasis on the effect of CLA on adipose tissue (measured by biopsy) and certain genes.

HOMA was calculated in 13 of the 20 studies in the assessment (Bonet Serra *et al*., 2008; Hermann *et al*., 2009; Lambert *et al*., 2007; Larsen *et al*., 2006; Laso *et al*., 2007; Moloney *et al*., 2004; Norris *et al*., 2009; Racine *et al*., 2010; Raff *et al*., 2008; Syvertsen *et al*., 2006; Taylor *et al*, 2006; Watras *et al*., 2006 and Whigham *et al*., 2004). No authors reported transforming data for HOMA logarithmically, with the possible exception of Lambert *et al*., (2007)[9]. All studies describing QUICKI also describe HOMA.

Four studies reported a decrease (not statistically significant) in HOMA:

- Lambert *et al*., (2007) in overweight but not obese men and women with metabolic syndrome

- Laso *et al.*, (2007) in overweight and marginally obese men and women

- Syvertsen *et al*., (2006) among overweight and obese men and women

- Whigham *et al*., (2004) during the very low calorie diet phase of the study only.

The one study to report a significant decrease in HOMA was Bonet Serra *et al*., (2008); a study of 8-19 year old children and adolescents (refer Section 3.6).

Nine studies reported an increase in HOMA (Herrmann *et al*., 2009; Larsen *et al*., 2006; Moloney *et al*., 2004; Norris *et al*., 2009; Racine *et al*., 2010; Raff *et al*., 2008; Taylor *et al*., 2006; Watras *et al*., 2006; and Lambert *et al*., 2007 among relatively lean and physically active men only). The reported increases in HOMA were statistically significant, i.e. an adverse finding, in Norris *et al*., (2009) and Moloney *et al*., (2004) only; the two studies involving subjects with type 2 diabetes.

Two studies reported no change in HOMA:

- Laso *et al*., (2007), in BMI >30

- Whigham *et al*., (2004), in the weight maintenance study phase.

[9] Lambert *et al*., (2007) used the following formula to calculate HOMA:
Resistance = insulin/($22.5e^{-\ln glucose}$)

HbA_{1c} measures are most meaningful in the monitoring of diabetic control. Five studies reported HbA_{1c} values, which gives an indication of average blood glucose over the last 8-12 weeks. Of these five studies, only Moloney *et al*., (2004) studied diabetics but Syvertsen *et al*., (2007) may have included some diet-controlled diabetics. Among the five studies, one reported a decrease, two an increase and two no difference in HbA_{1c} between subjects receiving CLA and controls. All measured effects were small (<1%) in relation to the value of <7% that is considered desirable for good diabetic control (Berven *et al*., 2000; Gaullier *et al*., 2004; Moloney *et al*., 2004; Risérus *et al*., 2002a and Syvertsen *et al*., 2007). The duration of the studies should be considered when interpreting HbA_{1c} measures; Moloney *et al*., (2004), Berven *et al*., (2000) and Risérus *et al*., (2002a) were short studies of 8, 12 and 12 weeks respectively, whilst the other two studies were of more than six months duration.

3.2 Effect of CLA (1:1) on glucose homeostasis in adults with diabetes or the metabolic syndrome

FSANZ attempted to group studies into those with subjects with diabetes, impaired glucose metabolism, or the metabolic syndrome. The description of participants in the studies was not adequate for clearly dividing the studies into groups and this is highlighted in Table 1 below. FSANZ acknowledges that the grouping of studies in Table 1 is somewhat crude.

Table 1: Summary of findings: 1:1 CLA isomer studies in adults with diabetes or the metabolic syndrome ordered according to dose

First author, year	CLA dose g/day (duration)	Final no. of subjects in the CLA study arm	Key subject characteristics	Method for measuring insulin sensitivity	Key results
Moloney, 2004	2.2 (56 days)	16	Overweight with stable, diet-controlled type 2 diabetes. Some subjects on anti-hypertensive medication. Gender breakdown not reported.	OGTT, fasting glucose and insulin concentrations, HOMA and QUICKI	Sig increase in HOMA and fasting glucose in CLA compared to controls who took an oil blend with 45% SFA to mimic western diet
Risérus, 2002a	2.4 (84 days)	19	Overweight or obese men with the metabolic syndrome.	Clamp technique	NS
Larsen, 2006	2.6 (365 days)	38	Overweight or obese men and women. Included diet-treated type 2 diabetics and subjects with treated simple hypertension.	HOMA	NS
Laso, 2007	3.0 (84 days)	20	Overweight or obese men and women with the metabolic syndrome. Unclear if diet-controlled diabetics included.	HOMA	NS
Risérus, 2001	3.1 (28 days)	14	Abdominally obese men, some of whom were taking blood pressure lowering medication.	Fasting glucose and insulin concentrations only	NS
Syvertsen, 2006	3.4 (182 days)	24	Excluded type 1 and untreated type 2 diabetics. Authors acknowledge some subjects insulin resistant at baseline (data not shown)	Clamp technique, HOMA and QUICKI	NS

First author, year	CLA dose g/day (duration)	Final no. of subjects in the CLA study arm	Key subject characteristics	Method for measuring insulin sensitivity	Key results
Smedman, 2001	4.2 (84 days)	24	No subject inclusion or exclusion criteria provided. Men and women. BMI range 19.1-34.5 kg/m^2. SBP range 94-170 mmHg. Waist range 64-114 cm.	Fasting glucose and insulin concentrations only	NS
Norris, 2009‡	6.6 (2 x 112 days with 28 day washout)	35	Post-menopausal women taking oral-hypoglycaemic agents to control type 2 diabetes.	HOMA	Sig increase in HOMA in CLA compared to control; safflower oil control group had sig ↓ in fasting glucose

More detail of results is provided in Table B1 at the rear of this report. ‡ crossover design. NS: Not significant, (i.e. p>0.05). SBP = systolic blood pressure. SFA = saturated fatty acids

Two of the eight studies in Table 1 above were designed to test the effect of CLA in diabetics (Moloney *et al.,* 2004 and Norris *et al.,* 2009). These two studies found statistically significant adverse changes in HOMA, i.e. subjects taking CLA were more resistant to insulin at the end of the studies compared to controls. Differences between the two studies (including but not limited to the dose of CLA, type of control oil, age and body weight of subjects, medication use, and baseline fasting glucose concentrations) make them difficult to compare. The crossover design of Norris *et al.,* (2009), with 35 subjects acting as their own control and using a relatively high dose of CLA (6.6 g), has more power than the other studies in Table 1. Norris *et al*., (2009) found CLA significantly decreased BMI and fat mass with no change in lean body mass (refer **SD2**). It might be expected that a significant change in fat mass would lead to improved glucose homeostasis. However no effect on fasting glucose or fasting insulin concentrations was recorded in the CLA group. The authors suggested that the weight loss recorded may not have been sufficient to improve markers of glucose homeostasis. The significant results of two well conducted studies (Norris *et al.,* (2009) and Moloney *et al.,* (2004)) are of particular interest and raise safety concerns for people with type 2 diabetes.

Risérus *et al*. (2002a) used the 'gold standard' clamp technique and reported similar changes in insulin sensitivity in overweight and obese men with metabolic syndrome taking 3.4 g CLA compared to olive oil. Syvertsen *et al.* (2007) also used the clamp technique in overweight participants and reported similar changes between CLA and control after six months.

No statistically significant effects of CLA on glucose or insulin concentrations were found in any of the studies in Table 1 except for the two studies that involved diabetics only (i.e. Moloney *et al*., 2004 and Norris *et al.,* 2009). Markers of glucose homeostasis may respond differently depending on the status of the subjects.

However, the description of participants in the studies was not adequate for clearly dividing the studies into groups with diabetes, impaired glucose metabolism, or the metabolic syndrome. An additional source of methodological variation among the studies is the variety of glucose homeostasis markers reported and the small number of studies that have used the 'gold-standard' method of the euglycaemic clamp.

3.3 Effect of CLA (1:1) on glucose homeostasis in healthy, overweight or obese subjects

Five studies remain where 'healthy' overweight or obese adult subjects were recruited (Berven *et al*., (2000), Gaullier *et al.,* (2004), Taylor *et al*., (2006), Watras *et al*., (2006) and Herrmann *et al*., (2009) (results are provided in Table B2). The authors of Taylor *et al*., (2006) reported the aim of their study "Was to assess the efficacy of CLA as an aid to weight loss and its effect on cardiovascular risk factors... [including] insulin sensitivity". The authors found no significant effects of CLA on glucose homeostasis. The study by Herrmann *et al*., (2009) was a crossover study designed to evaluate isomer specific CLA effects in human adipose tissue (via biopsy) and to investigate whether CLA affects depend on certain genes. Herrmann *et al*., (2009) found no significant effect of CLA (1:1) on HOMA compared to a linoleic acid control (FSANZ notes that three subjects with elevated glucose levels 'during most or all interventions' were excluded from the analysis). There were a total of four phases in this crossover study (refer Appendix 2 for additional discussion). The other studies shown in Table B2 collected insulin and glucose concentrations as secondary outcome measures. The design of these studies was variable (refer Table A). None of these studies reported any statistically significant effects of CLA on glucose homeostasis.

3.4 Effect of CLA (1:1) on glucose homeostasis following initial weight reduction

Improvements to glucose homeostasis are expected following weight loss (IDF (2006), Risérus *et al*., (2001)); therefore, FSANZ separately grouped weight regain studies to facilitate interpretation of results for this assessment. Three studies can be grouped into this classification (Kamphuis *et al*., (2003), Larsen *et al.,* (2006) and Whigham *et al.,* (2004)) (refer Table B3). The three studies provided values for HOMA and report no statistically significant results.

3.5 Effect of CLA (1:1) on glucose homeostasis in healthy adult subjects with normal body weight

Four of the 20 studies in this assessment involved subjects with a healthy body weight (BMI >20 and ≤25 kg/m^2) (Lambert *et al*., (2007), Noone *et al.*, (2002), Raff *et al*., (2008) and Smedman and Vessby (2001)). The differences in methodology and design of these four studies limit comparisons that can be made. The lean subjects in the study by Lambert *et al*., (2007) were exercising and this study involved an OGTT whereas others did not. Raff *et al*., (2004) excluded subjects who did more than 10 hours of heavy exercise per week, indicating some level of vigorous exercise may have been undertaken, and the young men in that study had approximately half of their daily energy intake substituted with test food. The study by Smedman and Vessby (2001) included not only lean but also overweight and obese subjects, and possibly included subjects with the metabolic syndrome amongst those (refer also Table 1 above). Combined, the studies provide limited data to assess the effect of CLA on glucose homeostasis in healthy adults with normal body weight (refer Table B4). The authors of these studies reported non-significant effects of CLA on glucose homeostasis.

3.6 Effect of CLA (1:1) on glucose homeostasis in overweight or obese children and adolescents

Two published blinded randomised control trials of CLA in children and adolescents were identified (Bonet Serra *et al*., 2008, Racine *et al.,* 2009) (results are provided in Table B5). The trial by Bonet Serra *et al*., included 39 obese children and adolescents aged 8-19 years given liquid yoghurt containing 3 g of CLA per daily serving or the same drink with nothing added. The less than ideal control of this study is noted, as was the case for the adult study by Laso *et al* (2007) mentioned previously. After 16 weeks the CLA group had a reduction in mean plasma glucose concentration (0.24 mmol/L) compared to a small increase in controls (0.14 mmol/L). The CLA group also had a reduction in HOMA, an indication that they had become less resistant to insulin. The trial by Racine *et al*., (2010) ran for approximately 7 months and involved 6-10 year old pre-pubertal children. The children took 2.4 g CLA or sunflower oil placebo in chocolate milk. No significant differences in fasting glucose, insulin or HOMA between CLA and control groups were found after 7 months.

The Applicant also provided an as yet unpublished report by the same researchers (Bonet Serra *et al*., unpublished) with similar findings, but no clear statistical difference between group comparisons, and no information on the formulation of the CLA used. Therefore, this report was not considered in this assessment.

4. Discussion

The Applicant is seeking to add CLA to food and therefore studies that administered CLA to subjects in food vehicles are of particular interest. However, grouping studies in this assessment according to the method in which CLA was administered cannot be justified because only four of the 20 1:1 isomer studies administered CLA in food form (Bonet Serra *et al*., 2008, Laso *et al*., 2007, Raff *et al*., 2008 and Racine *et al*., 2010).

The variability in study design and subject characteristics has been highlighted in this assessment. FSANZ attempted to group like studies (based primarily on subject characteristics), but because of variability in the studies, somewhat crude grouping was sometimes all that could be achieved.

Approximately half the studies in the assessment that reported the difference between CLA and control groups was not statistically significant, did not report any measure of variability around their results (such as the inter-quartile range or the standard deviation). From the other half that did report variability, the standard deviation varied from 0.2 to 0.8 mmol/L for glucose and 8.8 to 80.6 pmol/L for insulin in studies of adults (although the standard deviation is not a robust descriptor for parameters that are not normally distributed such as glucose and insulin). This further illustrates the great variability in the characteristics of the subjects that were included in the studies and may also explain the variability in results (either an increase or a decrease in their parameters of various magnitudes). With few exceptions, the differences in glucose between the groups were small (0- 0.2 mmol/L) between the CLA and control groups compared to the normal (non-diabetic) maximum of <8 mmol/L.

Two studies are of particular interest (Norris *et al.,* (2009) and Moloney *et al.,* (2004)). These studies were well conducted and were the only two studies of the 20 studies in the assessment that involved diabetics; they also reported significant adverse results. These two studies raise safety concerns about the effects of CLA on people with type 2 diabetes.

Caution is required when interpreting the results of the majority of the studies in this assessment. The relationship between glucose homeostasis and dietary factors is complex and not as well characterised as the relationship between fatty acid intake and blood lipid profile. The absence of a benchmark effect size combined with the small sample sizes and heterogeneous populations studied makes it very difficult to draw inferences from the available data.

5. Conclusion

Few studies testing the 1:1 isomer ratio have assessed the effect of CLA on insulin sensitivity directly using the 'gold standard' clamp technique. The two studies using this technique have reported no significant effect of CLA (Risérus *et al*., 2002a and Syvertsen *et al*., 2006). Few studies have measured glucose tolerance using the OGTT, and the inconsistent results from these studies may be related to the variation in health and weight status of participants in the trials (Lambert *et al*., 2007 and Moloney *et al*., 2004). A larger number of studies have estimated insulin resistance via the HOMA index. The majority reported no statistically significant effect of CLA on HOMA. Significant adverse effects of CLA were reported via increased estimates of HOMA in two studies involving diabetics only (Moloney *et al*., 2004 and Norris *et al*., 2009).

Indicators of glucose homeostasis may respond differently depending on the health status of the subjects. However, the description of participants in the studies was not adequate for clearly dividing the studies into groups with diabetes, impaired glucose metabolism, metabolic syndrome or normal metabolism. There were a number of instances where studies described their participants, for example, as healthy but also had exclusion criteria such as BMI<35kg/m^2. Additional mean baseline data then indicated elevated blood pressure or other characteristics that could define the presence of metabolic syndrome in at least some of the participant population. The variation in body weight or proportion with normal or abnormal glucose metabolism among the studies may account for some of the variation in results between studies. Consequently it is unclear which of the studies described above could be extrapolated to the population without metabolic syndrome. An additional source of methodological variation among the studies is the variety of glucose homeostasis markers reported and the small number of studies that have used the 'gold-standard' method of the clamp technique.

The two studies of CLA in children and adolescents do not allow any conclusions to be drawn for this group.

The available data raises questions but does not permit a conclusion about the effect of CLA on glucose homeostasis in the general population. Two well conducted studies raise safety concerns about the effects of CLA on people with type 2 diabetes.

Appendix 1

Table A: Summary of participant details and protocols in included studies of CLA 1:1 isomer ratio

First Author, Year	Final n (m/f)	Dropouts	Physical State BMI (kg/m^2)	Mean baseline glucose mmol/L	Age (years)	Duration (days)	CLA only (g/d)	Placebo, Dose (g/d)	Dietary/physical activity assessment	Notes
Berven *et al*., 2000	47 (30/17)	5 dropouts, 8 exclusions, 2 adverse events (possibly CLA-related)	Overweight or obese BMI 27.5-39	ND	≥18	84	3.4	4.5 olive oil	Diet & physical activity were not reported	Standard fasting glycated haemoglobin measured
Bonet Serra *et al*., 2008	39 (13/26)	Not stated	>95 centile for age	ND	8-19	112	3.0 in yoghurt drink	Yoghurt drink (2 x 100 g/d) without additions	Participants were given a diet & physical activity journal. Energy intake and physical activity fell in both groups during the study.	Overweight and obese children and adolescents Calculated HOMA
Gaullier *et al*., 2004	157 (31/149, at start of study)	23 dropouts – 10 due to adverse events, 1 due to pregnancy, 12 unspecified	Overweight BMI 25-30	All= 5.1	18-65	365	3.6 (FFA) 3.4 (TAG)	4.5 olive oil	Diet & activity were assessed by questionnaires at 0, 6 & 12 months	All groups reduced their energy intake from month 0 to month 12, but the relative change between CLA & control was NS. NS difference in exercise in CLA vs. control
Herrmann *et al*., 2009‡	34 (34/0)	4; one due to illness and three because they showed elevated fasting glucose levels	Overweight (mean BMI 26), abdominally obese (mean waist 102 cm)	ND	45-68	4 x 28 + 42 day washout btw each phase	3.4	3.2 linoleic from safflower oil	ND	Calculated HOMA

First Author, Year	Final n (m/f)	Dropouts	Physical State BMI (kg/m^2)	Mean baseline glucose mmol/L	Age (years)	Duration (days)	CLA only (g/d)	Placebo, Dose (g/d)	Dietary/physical activity assessment	Notes
Kamphuis *et al*., 2003	54 (26/28)	6 dropouts; 1 for illness, 1 due to medication use, 4 for motivational reasons	Overweight BMI 25-30	At Wk -3 (before VLCD) Cont 1.8=5.3 Cont 3.6=5.3 CLA 1.4=5.0 CLA 2.7= 5.0	20-50	91	1.4 / 2.7	1.8/3.6 oleic acid	Subjects placed on VLCD for 3 weeks prior to intervention resulting in a mean weight loss of 6.9%. Physical activity was monitored by accelerometer but only in the 2.7 g CLA and 3.6 g control groups.	Standard fasting insulin
Lambert *et al.,* 2007	62 (26/38[a])	2 dropouts	Regularly exercising (3 or more time per week) BMI **<25**	Cont M=5.0 Cont F=4.8 CLA M=5.0 CLA F=4.8	21-45	84	2.6	3.9 high oleic acid sunflower oil	Physical activity records throughout study quantified as metabolic equivalents. 3 x 4 day diet record.	OGTT used to measure insulin & glucose
Larsen *et al*., 2006	83 (36/47)	18 dropouts wk 26 and a further 6 by wk 52 (6 withdrawals due to adverse events amongst those dropouts)	Healthy, overweight or obese diabetics BMI 28-35	Cont=4.9 CLA=4.9	18-65	365	3.6	4.5 olive oil	Subjects placed on LCD for 8 weeks prior to intervention, ≥ 8% weight loss required for participation in treatment. 3 x 3 day diet records.	BW, BFM, and LBM were higher in controls at baseline; these were included as covariates in the final analysis. Glucose & insulin fasting blood test

First Author, Year	Final n (m/f)	Dropouts	Physical State BMI (kg/m^2)	Mean baseline glucose mmol/L	Age (years)	Duration (days)	CLA only (g/d)	Placebo, Dose (g/d)	Dietary/physical activity assessment	Notes
Laso *et al.,* 2007	43 (33/10)	2 lost to follow up and 15 lost to protocol violation	Metabolic syndrome, overweight or obese BMI 25-35	Con BMI<30 = 4.8 Con BMI **≥**30 **= 5.3** CLA BMI<30 = 5.7 CLA BMI ≥30 = 5.1	35-65	84	3.0 in skim milk	Non-fortified skim milk	6 3-day diet records & 3 FFQ. Subject's results were excluded if daily energy intake varied by more than 10%. Physical activity monitored 'throughout study' via questionnaire.	Subjects had waist circum > 102cm (m) and >88cm (f) and had to fulfil at least two criteria for metabolic syndrome
Moloney *et al*., 2004	32 (ND)	No dropouts	Type 2 diabetes, overweight BMI **~ 30**	Cont=7.3 CLA=7.3	50-70	56	2.2	3 soya bean & palm oil blend	4-day food record at baseline & before completion	Also measured QUICKI & ISI OGTT used to measure insulin & glucose
Noone *et al*., 2002	51 (18/33)	No dropouts	Sedentary BMI <25	Cont=5.0 CLA=4.9	32±10	56	1.9 (1:1 blend)	Linoleic acid (amt not specified)	No attempts at assessing or controlling diet were reported. To be eligible, subjects had to do < 90 minutes strenuous exercise/week.	Only included group who used 1:1 isomeric blend in Table B Standard fasting insulin & glucose blood test
Norris *et al*., 2009[‡]	35 (0/35)	20 dropouts; 3 due to time commitment, 3 to GI complaint, 6 for unrelated health concerns, 2 glycaemia worsened, 3 unable to obtain venous access, and 3 lost to follow up	Type 2 diabetes, post-menopausal obese BMI **>30**	Cont=4.8 CLA= 4.9	59.7 ±7.3	2 x 112 + 28 day washout	6.6	8 safflower oil	3 day diet & activity records kept on 4 occasions during the study	Subjects taking oral hypoglycaemic agents Standard fasting insulin & glucose blood test

First Author, Year	Final n (m/f)	Dropouts	Physical State BMI (kg/m^2)	Mean baseline glucose mmol/L	Age (years)	Duration (days)	CLA only (g/d)	Placebo, Dose (g/d)	Dietary/physical activity assessment	Notes
Racine *et al.,* 2010	53 (31/22)	10 chose not to participate, 7 dropouts, 2 did not qualify for data analysis	Overweight & obese children BMI ≥ 85th percentile	Cont=5.0 CLA=5.0	6-10	*183*	2.4 in chocolate milk	3.0 sunflower oil in chocolate milk	Ad libitum diet. Dietary advice provided at start of study.	Overweight & obese children
Raff *et al*., 2008	38 (38/0)	9; 5 from CLA group and 4 from control group	Healthy Mean BMI = 22	Cont=4.7 CLA=4.8	19-35	*35*	4.6 added to low CLA butter	115g low CLA butter	Subjects replaced1/2 (6.9MJ/d) their diet with test foods (butter) used in food such as bread rolls and cake so that both groups had similar total fat intake without increasing the total fat content of their diets	Standard fasting insulin & glucose blood test.
Risérus *et al*., 2001	24 (24/0)	1 dropout	Obese BMI 27-39	Cont=5.3 CLA=4.6	39-64	28	3.1	4.2 olive oil	Diet interviews at baseline to estimate dietary CLA intake, but no formal assessment throughout study.	Standard fasting insulin & glucose blood test
Risérus *et al*., 2002a	57 (57/0)	3 dropouts	Metabolic syndrome, abdominally obese BMI 27-39	Cont=5.7 CLA=5.9	43-63	84	2.4 (1:1 isomers) or 2.6 (t10,c12)	3.4 olive oil	3-day weighed food record kept at week 0 & 8	Only included group who used 1:1 isomeric blend in Table B Used euglycemic hyperinsulinemic clamp method
Smedman, 2001	50 (25/25)	3 exclusions due to poor compliance	Healthy BMI 19-35	Cont=4.7 CLA=5.9	23-63	84	4.2	4.2 olive oil	3 day weighted diet record kept at baseline, middle & end of study.	Standard fasting insulin & glucose blood test

First Author, Year	Final n (m/f)	Dropouts	Physical State BMI (kg/m^2)	Mean baseline glucose mmol/L	Age (years)	Duration (days)	CLA only (g/d)	Placebo, Dose (g/d)	Dietary/physical activity assessment	Notes
Syvertsen *et al*., 2006	41 (13/28)	8 dropouts	Overweight and obese BMI 28-32	Cont=5.4 CLA=5.4	27-64	182	3.4	4.5 olive oil	Diet and activity was assessed by questionnaires at 0 & 6 months	Used euglycemic hyperinsulinemic clamp method
Taylor *et al*., 2006	40 (40/0)	No dropouts	Healthy, obese BMI **33 ± 3**	Cont=5.2 CLA=5.1	35-60	84	3.2	4.5 olive oil	No measures of diet or physical activity reported.	Standard fasting insulin & glucose blood test
Watras *et al*., 2006	40 (8/32)	8 dropouts	Overweight BMI 25-30	Cont=4.8 CLA=4.9	18-44	182	3.2	4 safflower oil	7-day physical activity & 3 day diet records kept at baseline & study end.	
Whigham *et al.,* 2004	48 (at wk 28 of study) (15/33)	15 dropouts (6 too busy; 1 lost to follow-up; 3 adverse events; 1 pregnant; 2 could not take VLCD; 1 thyroid levels; 1 lack of commitment)	Obese BMI Mean: 32 Range: 25-37	ND	18-50	Controlled wk 0-28 only	5.6	7.5 high oleic sunflower oil	Diet & physical activity diaries submitted monthly. Initial VLCD (12 wks) followed by maintenance diet (16 wks) that many subjects found difficult to adhere to. Phase 3 (wk 28-52) all participants took CLA.	Authors did not specifically report results from baseline comparisons. Standard fasting insulin & glucose

Notes:
[a] The final gender composition was not reported.
‡ Crossover study design
ND = no data provided.

Table B: Selected results from CLA studies of 1:1 isomer ratio explanatory notes

This assessment includes 20 studies where CLA with *c9t11* and *t10, c12* isomers were examined in a 1:1 ratio (refer Table A). To facilitate discussion and analysis of results, the studies have been grouped into five tables. **Two studies appear in more than one table** (Larsen *et al*., 2006, Smedman & Vessby, 2001). The study by Larsen *et al.,* (2006) appears in both Table B1 and B3 as the study included subjects with diet-treated diabetes or possibly metabolic syndrome (though the number of such subjects is not defined) and all subjects underwent an 8 week low calorie diet. The study by Smedman & Vessby (2001) appears in both Table B1 and Table B4. In that study, subjects had BMI ranging from 19.1 to 34.5 kg/m^2; systolic blood pressure ranging from 94-180mmHg; and waist circumference measures ranging from 64-114cm. No inclusion or exclusion criteria were reported by Smedman & Vessby (2001) making this study difficult to categorise under the grouping of results devised by FSANZ for this assessment.

Table B provides summarised mean change in reported results from baseline, with statistical comparisons (*P*-values) between CLA and control groups. The arrows in the *P*-value column indicate the direction of change in the mean of the CLA group relative to the control such that ↓ indicates the CLA group experienced a relative decrease, ↑indicates the CLA group experienced a relative increase, and – indicates both the CLA group and the control group experienced the same magnitude of change in the same direction. Insulin and glucose are reported as concentrations in blood after an overnight fast.

Acronyms: f – females; HbA_{1c} – glycosylated haemoglobin. HOMA – homeostasis model assessment of insulin resistance; ISI – insulin sensitivity index; ITT – intention to treat analysis; m – males; ND – no data, where this replaces a standard deviation it indicates the mean differences were not reported in the paper and needed to be calculated; NS – not statistically significant (*P* >0.05); OGIS – oral glucose insulin sensitivity (from OGTT); OGTT – oral glucose tolerance test; QUICKI – quantitative insulin sensitivity index; SD – standard deviation.

Table B: Selected results from studies of CLA with 1:1 isomer ratio

Table B1: Studies in adults with diabetes or the metabolic syndrome (8 studies)
Table B2: Studies in healthy, overweight or obese adults (5 studies)
Table B3: Studies in adults following initial weight reduction (3 studies)
Table B4: Studies in healthy adults with normal body weight (4 studies)
Table B5: Studies in overweight or obese children and adolescents (2 studies)

Table B1: Studies in adults with diabetes or the metabolic syndrome

Paper	Group	Δ Insulin from baseline (pmol/L±SD)	Change between groups P – value	Δ Glucose from baseline (mmol/L ± SD)	Change between groups P - value	Δ HbA_{1c} from baseline (%)	Change between groups P - value	Δ HOMA-IR from baseline	Change between groups P - value	Notes:
Larsen *et al.*, 2006	CLA	12.9±18.9	↑NS (0.22)	0.16±0.73	↑NS (0.20)	ND		3.01±4.63	↑NS (0.12)	Subjects were on a LCD for 8 wk to lose ≥ 8% BW. Included diet-treated diabetics (n not reported) as well as subjects with treated simple hypertension (n not reported).
	Con	6.66±24.2		-0.40±0.61				1.10±5.72		
Laso *et al.*, 2007	CLA (BMI>30)	14.58±ND	↑NS	-0.20±ND	↓NS	ND	ND	0.1±ND	NS	Metabolic syndrome. Also note excluding diabetics treated with insulin or drugs only. Number of diet-treated diabetics not reported.
	Con (BMI>30)	-0.69±ND		-0.03±ND				0.1±ND		
	CLA (BMI≤30)	2.08±ND	↓NS	-0.34±ND	↓NS			-0.1±ND	NS	
	Con (BMI≤30)	10.42±ND		-0.19±ND				0.2±ND		
Moloney *et al*., 2004	CLA	5.74±ND	↑NS	0.46±ND	↑<0.05	-0.30±ND	↓NS	0.54	↑0.05	Stable, diet-controlled type 2 diabetics; some on antihypertensive medication. NS in QUICKI. Lower OGIS (*P*=0.05) and ISI (*P*<0.050).
	Con	-4.26±ND		-0.24±ND		-0.14±ND		-0.41		

Paper	Group	Δ Insulin from baseline (pmol/L±SD)	Change between groups P – value	Δ Glucose from baseline (mmol/L ± SD)	Change between groups P - value	Δ HbA_{1c} from baseline (%)	Change between groups P - value	Δ HOMA-IR from baseline	Change between groups P - value	Notes:
Norris *et al.*, 2009 (cross-over study)	CLA (CLA-Con)	-7.64±15.97‡	–NS (0.46)	0.28±0.56	↑0.011	ND		0.1±1.0	↑0.05	Type 2 diabetics taking oral hypoglycaemic agents.
	Con (CLA-Con)	-7.64±18.06‡		-0.61±0.61				-1.3 ±0.8		
	CLA (Con-CLA)	9.72±13.89‡		0.61±0.50				1.3±0.9		
	Con (Con-CLA)	-11.81±12.50‡		-1.05±0.44				-0.8 ± 1.2		
Risérus *et al.*, 2001	CLA	4.93±ND	↓ NS	0.25±ND	↓ NS	ND		ND		Metabolic syndrome; some subjects taking antihypertensive medication (n not reported).
	Con	13.75±ND		0.46±ND						
Risérus *et al.*, 2002a	CLA	4.80±19.20	↓ NS	0.01±0.30	↑ NS	0.04	-NS	ND		Metabolic syndrome. Six subjects of 57 with fasting glucose 7.0-7.2 indicating mild diabetes. NS changes in insulin sensitivity as assessed by hyperinsulinaemic euglycaemic clamp.
	Con	5.52±23.3		-0.14±0.24		0.04				

Paper	Group	Δ Insulin from baseline (pmol/L±SD)	Change between groups P – value	Δ Glucose from baseline (mmol/L ± SD)	Change between groups P - value	Δ HbA_{1c} from baseline (%)	Change between groups P - value	Δ HOMA-IR from baseline	Change between groups P - value	Notes:
Smedman & Vessby, 2001	CLA	1.16	↑ NS 0.6	0.11	↑ NS 0.053	ND		ND		
	Con	-2.3		-0.66						
Syvertsen et al., 2006	CLA	-0.75^{+}	↑ NS (0.39)$^{+}$	-0.08^{+}	↑ NS (0.6)$^{+}$	0^{+}	↑ NS	-0.25* and -0.08^{+}	↓NS(0.58)* And ↑ NS (0.35)$^{+}$	*for n=83 in main study $^{+}$for n=41 in clamp study (subset of 83 in main study) NS change in QUICKI or results from the euglycaemic clamp in CLA vs. control
	Con	-16.0^{+}		-0.25^{+}		-0.2^{+}		-0.23* and -0.53^{+}		

Table B2: Studies in healthy, overweight or obese adults

Author, year	Group	∆Insulin from baseline (pmol/L ±SD)	Change between groups p value	∆Glucose from baseline (mmol/L ±SD)	Change between groups p value	∆ HbA_{1c}	Change between groups p value	∆ HOMA-IR from baseline‡	Change between groups p value	Notes
Berven *et al*. 2000	CLA	ND		ND		0.1±0.4	NS (0.43)	ND		
	Con					0.1±0.3				
Gaullier *et al*. 2004	CLA 3.4	Values not reported	NS	0.08±0.60	- NS	0.21±0.23	↑ NS	ND		
	CLA 3.6			-0.05±0.44		0.14±0.22				
	Con			-0.10±0.44		0.16±0.20				
Herrmann *et al.*, 2009 (cross-over study)	CLA phase compared to control phase	ND		ND		ND		0.2	↑ NS (0.7)	P value is for the difference between the control phase and 3 phases in which either the 1:1 ratio, or each isomer, was given
Taylor *et al*. 2006	CLA	1.60±8.75	↑ NS (0.50)	0.20±0.80	↓ NS (0.51)	ND		0.80±3.2	↑ NS (0.50)	
	Con	0.49±15.41		0.30±0.80				0.20±6.7		
Watras *et al.* 2006	CLA	14.58±80.56	↑ NS	0.16±0.27	↑ NS	ND		0.60±2.8	↑ NS	
	Con	-4.16±43.06		0.33±0.33				0.00±1.4		

‡ Except for Herrmann *et al.,* (2009) where the difference in HOMA at the end of the intervention phase compared to the control phase is shown.
ND – no data, where this replaces a standard deviation it indicates the mean differences were not reported in the paper and needed to be calculated.
NS – not statistically significant (*P* >0.05). FSANZ converted blood glucose values reported in mg/dL to mmol/L by multiplying by 0.0555.
FSANZ converted insulin concentrations reported in µIU/mL to pmol/L by multiplying by 6.945.

Table B3: Studies in adults following initial weight reduction

Author, year	Group	∆Insulin from baseline (pmol/L ±SD)	Change between groups p value	∆Glucose from baseline (mmol/L ±SD)	Change between groups p value	∆ HbA_{1c}	Change between groups p value	∆ HOMA-IR from baseline	Change between groups p value	Notes
Kamphuis *et al*.2003	CLA 1.4	12.5±ND	↑ NS	0.40 ±ND	↑ NS	ND		ND		
	Con 1.8	6.95±ND		0.30 ±ND						
	CLA 2.8	14.58±ND	↑ NS	0.20 ±ND	- NS					
	Con 3.6	22.22±ND		0.20 ±ND						
Larsen *et al.*, 2006	CLA	12.9±18.9	↑NS (0.22)	0.16±0.73	↑NS (0.20)	ND		3.01±4.63	↑NS (0.12)	Subjects were on a LCD for 8 wk to lose ≥ 8% BW. Included diet-treated diabetics (n not reported) as well as subjects with treated simple hypertension (n not reported).
	Con	6.66±24.2		-0.40±0.61				1.10±5.72		
Whigham *et al*. 2004	CLA (wk 0-12)	ND	↓ NS	ND	↑ NS	ND		-0.36±ND	↓ NS	CLA subjects have significantly higher serum glucose compared to placebo subjects at week 2, but the differences were NS at any other time. Week 0-12 subjects on VLCD. Week 12-28 subjects on maintenance diet, with report that the diet was difficult to adhere to.
	Con (wk 0-12)	ND		ND				-0.30±ND		
	CLA (wk 12-28)	ND	↑ NS	ND	↑ NS			0.13±ND	- NS	
	Con (wk 12-28)	ND		ND				0.13±ND		

Table B4: Studies in healthy adults with normal body weight

Paper	Group	Δ Insulin from baseline (pmol/L±SD)	*Change between groups P* – value	Δ Glucose from baseline (mmol/L ±SD)	*Change between groups P* - value	Δ HbA_{1c} from baseline (%)	*Change between groups P* - value	Δ HOMA-IR from baseline	*Change between groups P* - value	Notes:
Lambert *et al*., 2007	CLA M	ND		ND		ND		0.01±ND	↑ NS	Concurrent exercise intervention in healthy body weight subjects. QUICKI 30 minute insulin/glucose increment and fasting glucose/insulin ratio were NS between CLA and Con. OGTT insulin concentration was lower in women on CLA than control (p=0.04)
	Con M							0.07±ND		
	CLA F							-0.05±ND	↓ NS	
	Con F							0.03±ND		
Noone *et al*., 2002	CLA	-11.60±ND	↓ NS	-0.09±ND	↓ NS	ND		ND		
	Con	2.85±ND		0.24±ND						
Raff *et al.*, 2008	CLA	7.57	↑ NS	0.18	↓ NS	ND		2.06	↑ NS	Both groups were on a high saturated fatty acid diet, which may have masked CLA effect
	Con	2.87		0.34				0.6		
Smedman & Vessby, 2001	CLA	8.05±ND	↑NS (0.60)	0.11±ND	↑NS (0.053)	ND		ND		This study included subjects with normal BW, overweight and obese (BMI ranging from 19.1-34.5) but numbers in each category not given.
	Con	-15.97±ND		-0.06±ND						

Table B5: Studies in overweight or obese children and adolescents

Paper	Group	Δ Insulin from baseline (pmol/L±SD)	*Change between groups* *P* – value	Δ Glucose from baseline (mmol/L ±SD)	*Change between groups P* - value	Δ HbA_{1c} from baseline (%)	*Change between groups P* - value	Δ HOMA-IR from baseline	*Change between groups* *P* - value	Notes:
Bonet-Serra *et al*., 2008	CLA	-13.2±34.03	↓ NS	-0.24±0.48	↓ <0.05	ND		-0.60±1.2	↓ <0.05	Study in children and adolescents. Control gp had no fatty acid added to yoghurt drink for comparison with CLA gp.
	Con	-0.69±29.17		0.14±0.52				-0.10±1.0		
Racine *et al*. 2010	CLA	4.17±30.56	↑ NS (0.6)	0.10±0.29	↑ NS (0.3)	ND		0.20±0.9	↑ NS (0.4)	Study in children. CLA delivered in chocolate milk.
	Con	0.28±46.53		0.02±0.22				-0.02±2.0		

FSANZ converted blood glucose values reported in mg/dL to mmol/L by multiplying by 0.0555.
FSANZ converted insulin concentrations reported in µIU/mL to pmol/L by multiplying by 6.945.

Appendix 2: Effect of other ratios of the two CLA isomers

The effects of ratios other than the 1:1 ratio of these CLA isomers on glucose homeostasis have been studied (Malpuech-Brugère *et al.*, 2004; Naumann *et al*. 2005; Risérus *et al*. 2002a; Risérus *et al.*, 2004a and Herrmann *et al*., 2009). The results of these studies are summarised in this assessment, although the data is not shown, because if only one of the two CLA isomers does affect glucose homeostasis, then this might be clearer in studies using a higher proportion of that isomer.

Risérus *et al*. (2002a) used the euglycaemic clamp technique in men with metabolic syndrome to study the effects of 3.4 g purified *t*10,*c*12 isomer and 1:1 isomer mixture (reported above) compared to olive oil control. The group receiving purified *t*10,*c*12 isomer had significantly increased insulin resistance (calculated as M) compared to the control group. The authors also reported an increase in fasting glucose as well as a decrease in insulin sensitivity in participants receiving purified *t10,c12*. The same group subsequently tested the effect of 3.0 g *c*9,*t*11 isomer against olive oil in obese men using the clamp technique and reported that insulin sensitivity was lower (i.e. insulin resistance increased) in the *c*9,*t*11 isomer group compared to olive oil (Risérus *et al*., 2004a). Herrmann *et al*., (2009) was a four-phase crossover study of 38 (final n = 34) abdominally obese men. Intervention periods, each lasting 28 days, were separated by a 42-day washout. The four interventions were 3.4 g of *c9,t11*, or *t10,c12,* or 1:1 mix of those isomers or a 3.2 g linoleic acid control. Three subjects with elevated fasting glucose were excluded from the analysis. HOMA did not change significantly during any intervention compared to control.

Malpuech-Brugère *et al.* (2004) compared two different doses (1.5 g and 3 g) of each of the two isomers separately to high oleic acid sunflower oil in moderately overweight, normolipidaemic adults for 18 weeks. All fats were given in a dairy drink. In a similar study, Naumann *et al.,* (2005) also compared 3 g of each isomer (>80% purity) separately to high oleic sunflower oil in drinkable yoghurts over 13 weeks in men and women with LDL-phenotype B. In the first study, changes in plasma insulin and glucose concentration were similar between the *c*9,*t*11 and *t10,c12* CLA groups and the control group (Malpuech-Brugère *et al,* 2004). In the second study, plasma glucose was higher (but not significantly so), in both CLA isomer groups and there was no difference in plasma insulin or HOMA between the CLA groups and control (Naumann *et al.,* 2005). Sluijs *et al*., (2010) examined a mix of the two isomers in a 4:1 ratio (higher amounts of the *c9,t11* isomer) and reported that there were small non-significant differences between the two groups (those receiving CLA had a smaller decrease in serum glucose and a smaller increase in serum insulin and HOMA compared to the control group).

Two studies were excluded from consideration (Figure 1) but are mentioned here because their results might be interpreted as indicating that the isomers of interest have no effect on glucose homeostasis. Medina *et al*. (2000) conducted a very tightly controlled study in which seventeen women lived in a metabolic suite with all food supplied by the facility throughout the study. Plasma insulin and glucose concentrations were similar between the CLA and sunflower oil groups. However, they used a CLA blend containing between16-24% of four different CLA isomers, including the two of current interest and so their results cannot be attributed to either of the isomers of current interest. Tricon *et al.* (2004) compared the effects of the *c*9,*t*11 isomer to those of the *t*10,*c*12 isomer. They report that plasma glucose was higher during the *t*10,*c*12 phases of the crossover study, but plasma insulin and insulin resistance/sensitivity as assessed by HOMA and QUICKI, were similar between the two isomers. As there was no control group, these results do not indicate what effect these isomers have compared to no CLA.

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